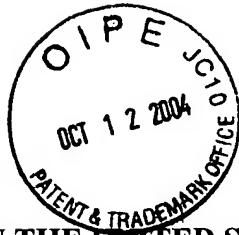


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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------|---|--------------------|
| Applicant | : | Curry, Kenneth |
| Appl. No. | : | 10/763,934 |
| Filed | : | January 23, 2004 |
| For | : | NOVEL AMINOINDANES |
| Examiner | : | unknown |
| Group Art Unit | : | 1614 |

REQUEST FOR CORRECTED PUBLICATION UNDER 37 C.F.R. § 1.221 (b)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is a request for corrected publication under 37 C.F.R. § 1.221 (b) for US 2004/0152670A1. The published document contains material errors that are not found in Applicant's records. Accordingly, the mistakes in the published document are believed to be due to PTO error. This request includes:

1. A listing of the alleged material errors made by the Office;
2. Marked up copies of the relevant pages of published application no. US 2004/0152670A1; and
3. An indication of where in the specification as filed the relevant text appears.



TRANSMITTAL

Applicant : Curry, Kenneth
App. No. : 10/763,934
Filed : January 23, 2004
For : NOVEL AMINOINDANES
Examiner : Unknown
Art Unit : 1614

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

October 5, 2004

(Date)

Che S. Chereskin

Che Swyden Chereskin, Ph.D., Reg. No. 41,466

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith for filing in the above-identified application are the following enclosures:

- (X) Request for Corrected Publication Under 37 C.F.R. § 1.221(b) in 4 pages with attached copy of published application noting requested corrections in red.
- (X) Return prepaid postcard.
- (X) Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Che S. Chereskin

Che Swyden Chereskin, Ph.D.

Registration No. 41,466

Agent of Record

Customer No. 20,995

(949) 760-0404

Appl. No. : 10/763,934
 Filed : January 23, 2004

| LOCATION IN PUBLISHED DOCUMENT | LOCATION IN SPECIFICATION AS FILED |
|--|------------------------------------|
| Page 1, paragraph [0005] – mGluR4 and mGluR6, the 4 and 6 should be subscript (line 17) | Page 2, line 12 |
| Page 1, paragraph [0008] – mGluR should be mGluR₁ (line 7) | Page 3, line 13 |
| Page 2, paragraph [0020] – sulfono, sulfono should be sulfono, sulfino (line 3) and $-(CH_2)_n$ -sulfono, $-(CH_2)_n$ - sulfino (line 5) | Page 5, lines 26 and 27 |
| Page 2, paragraph [0021] – sulfono, sulfono, should be sulfono, sulfino (line 3) | Page 6, line 2 |
| Page 3, paragraph [0027] – E. Haslarm should be E. Haslam (line 36) | Page 7, line 22 |
| Page 3, paragraph [0033] – n 1 should be n=1 (line 7) | Page 8, line 16 |
| Page 3, paragraph [0037] –acetyl sulfo, should be acetyl, sulfo (line 3) | Page 8, line 26 |
| Page 4, paragraph [0045] – rilonohydrogenphosphate should be monohydrogenphosphate (line 9) | Page 10, line 11 |
| Page 4, paragraph [0047] – aminonium should be ammonium (line 2) | Page 10, line 24 |
| Page 4, paragraph [0052] – Ecd should be Ed (line 16) | Page 11, line 26 |
| Page 6, paragraph [0074] – 50 should be 50° (line 6) | Page 15, line 5 |
| Page 7, paragraph [0079] – Formula m, should be Formula III (line 8) | Page 16, line 22 |

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 Filed : January 23, 2004

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|---|------------------|
| Page 8, paragraph [0096] – vifro should be vitro (line 1) | Page 20, line 21 |
| Page 8, paragraph [0099] – 1 MM should be 1mM (line 7) | Page 20, line 32 |
| Page 9, paragraph [0100] – IBM should be IBMX (line 8) | Page 21, line 7 |
| Page 9, paragraph [0101] – supematant should be supernatant (line 5) | Page 21, line 12 |
| Page 9, paragraph [0107] – [³ Cinositol should be [³ H] inositol (line 5) | Page 22, line 21 |
| Page 9, paragraph [0112] – (PBS)-Lic] should be (PBS)-LiCl (line 7) | Page 23, line 8 |
| Page 9, paragraph [0113] – IBEM should be IBMX (line 5) | Page 23, line 18 |
| Page 9, paragraph [0113] – IBM should be IBMX (line 9) | Page 23, line 21 |
| Page 10, paragraph [0118] – [3H] should be [³ H] (line 3) | Page 24, line 8 |
| Page 12, paragraph [0139] – missing sentence under Formulation 1 table to be inserted: The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities. | Page 29, line 26 |
| Page 12, paragraph [0140] – missing sentence under Formulation 2 table to be inserted: The components are blended and compressed to form tablets each weighing 665 mg. | Page 30, line 4 |
| Page 18, paragraph [0203] – (4x50 mL) should be (4x150 mL) (line 1) | Page 42, line 1 |

Appl. No. : 10/763,934
Filed : January 23, 2004

Conclusion

As this request for corrected publication under 37 C.F.R. § 1.221(b) is filed within two months of the publication date of the application (August 5, 2004), no fees are believed to be due. However, please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Oct. 5, 2004

By: Che I. Chereskin
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100404

NOVEL AMINOINDANES

FIELD OF THE INVENTION

[0001] This invention pertains to therapeutically active novel aminoindanes, a method for preparing the same, pharmaceutical compositions comprising the compounds and a method of treating diseases of the Central Nervous System (CNS) therewith.

BACKGROUND OF THE INVENTION

[0002] The acidic amino acid L-glutamate is recognized as the major excitatory neurotransmitter in the CNS. The receptors that respond to L-glutamate are called excitatory amino acid receptors. The excitatory amino acid receptors are thus of great physiological importance, playing a role in a variety of physiological processes, such as long-term potentiation (learning and memory), the development of synaptic plasticity, motor control, respiratory and cardiovascular regulation, and sensory perception.

[0003] Excitatory amino acid receptors are classified into two general types and both are activated by L-glutamate and its analogs. Receptors activated by L-glutamate that are directly coupled to the opening of cation channels in the cell membrane of the neurons are termed "ionotropic." This type of receptor has been subdivided into at least three subtypes, which are defined by the depolarizing actions of the selective agonists N-Methyl-D-aspartate (NMDA), α -Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and Kainic acid (KA).

[0004] The second general type of receptor is the G-protein or second messenger-linked "metabotropic" excitatory amino acid receptor. This second type is coupled to multiple second messenger systems that lead to enhanced phosphoinositide hydrolysis, activation of phospholipase D, increases or decreases in cAMP formation, and changes in ion channel function (Schoepp and Conn, *Trends in Pharmacological Science*, 14:13, 1993). Both types of receptors appear not only to mediate normal synaptic transmission along excitatory pathways but also to participate in the modification of synaptic connections during development and throughout life.

[0005] So far eight different clones of the G-protein-coupled mGluRs have been identified (Knopfel et al., 1995, *J. Med. Chem.*, 38, 1417-1426). These receptors function to modulate the presynaptic release of L-glutamate, and the postsynaptic sensitivity of the neuronal cell to L-glutamate excitation. Based on pharmacology, sequence homology and the signal transduction pathway that they activate, the mGluRs have been subclassified into three groups. The mGluR₁ and mGluR₂ receptors form group I. They are coupled to hydrolysis of phosphatidylinositol (PI) and are selectively activated by (RS)-3,5-dihydroxyphenylglycine (Brabet et al., *Neuropharmacology*, 34, 895-903, 1995). Group II comprises mGluR₃ and mGluR₄ receptors. They are negatively coupled to adenylate cyclase and are selectively activated by (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV; Hayashi et al., *Nature*, 366, 687-690, 1993). Finally, the mGluR₅, mGluR₆, mGluR₇, and mGluR₈ receptors belong to group III. They are also negatively coupled to adenylate cyclase and are selectively activated by (L)-2-amino-4-phosphonobutyric acid (L-AP4; Knopfel et al., 1995, *J. Med. Chem.*, 38, 1417-1426).

[0006] Agonists and antagonists of these receptors are believed useful for the treatment of acute and chronic neurodegenerative conditions, and as antipsychotic, antiemetic, analgesic, anxiolytic, antidepressant, and antiemetic agents. Antagonists and agonists of neural receptors are classified as selective for a particular receptor or receptor subtype, or as non-selective. Antagonists may also be classified as competitive or non-competitive. While competitive and non-competitive antagonists act on the receptors in a different manner to produce similar results, selectivity is based upon the observations that some antagonists exhibit high levels of activity at a single receptor type, and little or no activity at other receptors. In the case of receptor-specific diseases and conditions, the selective agonists and antagonists are of the most value.

[0007] Compounds such as L-Glutamic acid, Quisqualic acid and Ibotenic acid are known to act as non-selective agonists on the mGluRs, while selective ionotropic glutamate receptor agonists such as NMDA, AMPA and kainate have little effect on these receptors. Recently a few compounds without activity at the ionotropic glutamate receptors but with activity at the metabotropic receptors have been identified. These include trans-ACPD (trans-(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid), the partial agonist L-AP3 (L-2-amino-3-phosphonopropionic acid; Palmer, E., Monaghan, D. T. and Cotman, C. W. *Eur. J. Pharmacol.* 166, 585-587, 1989; Desai, M. A. and Conn, P. J. *Neuroscience Lett.* 109, 157-162, 1990; Schoepp, D. D. et al., *J. Neurochemistry*, 56, 1789-1796, 1991; Schoepp D. D. and Johnson B. G. *J. Neurochemistry* 53, 1865-1913, 1989), L-AP4 (L-2-amino-4-phosphonobutyric acid) which is an agonist at the mGluR₄ receptor (Thomsen C. et al., *Eur. J. Pharmacol.* 227, 361-362, 1992) and some of the isomers of CCG (2-(carboxycyclopropyl)glycines) especially L-CCG-I and L-CCG-II (Hayashi, Y. et al., *Br. J. Pharmacol.* 107, 539-543, 1992).

[0008] Very few selective antagonists at the mGluRs have been reported. However some phenylglycine derivatives, S-4CPG (S-4-carboxyphenylglycine), S-4C3HPG (S-4-carboxy-3-hydroxyphenylglycine) and S-MCPG (S- α -methyl-4-carboxyphenylglycine) have been reported to antagonize trans-ACPD-stimulated phosphoinositide hydrolysis and thus possibly act as antagonists at mGluR₁ and mGluR₂ subtypes (Thomsen, C. and Suzdak, P. *Eur. J. Pharmacol.* 245, 299, 1993).

[0009] Research directed towards mGluRs is beginning to show that mGluRs may be implicated in a number of normal as well as pathological mechanisms in the brain and spinal cord. For example, activation of these receptors on neurons can: influence levels of alertness, attention and cognition; protect nerve cells from excitotoxic damage resulting from ischemia, hypoglycemia and anoxia; modulate the level of neuronal excitation; influence central mechanisms involved in controlling movement; reduce sensitivity to pain; reduce levels of anxiety.

[0010] The use of compounds active at the mGluRs for the treatment of epilepsy is corroborated by investigations of the influence of trans-ACPD on the formation of convulsions (Sacaan and Schoepp, *Neuroscience Lett.* 139, 77, 1992) and that phosphoinositide hydrolysis mediated via mGluR is increased after kindling experiments in rats (Akiyama et al. *Brain Res.* 569, 71, 1992). Trans-ACPD has been shown to

mGluR₁ 7

Subscript

increase release of dopamine in the rat brain, which indicates that compounds acting on the mGluRs might be usable for the treatment of Parkinson's disease and Huntington's Chorea (Sacaan et al., *J. Neurochemistry* 59, 245, 1992).

[0011] Trans-ACPD has also been shown to be a neuro-protective agent in a medial cerebral artery occlusion (MCAO) model in mice (Chiamulera et al. *Eur. J. Pharmacol.* 215, 353, 1992), and it has been shown to inhibit NMDA-induced neurotoxicity in nerve cell cultures (Koh v, *Proc. Natl. Acad. Sci. USA* 88, 9431, 1991). The mGluR-active compounds are also implicated in the treatment of pain. This is proved by the fact that antagonists at the mGluRs antagonize sensory synaptic response to noxious stimuli of thalamic neurons (Eaton, S. A. et al., *Eur. J. Neuroscience*, 5, 186, 1993).

[0012] The use of compounds active at the mGluRs for treatment of neurological diseases such as senile dementia have also been indicated by the findings of Zheng and Gallagher, *Neuron* 9, 163, 1992 and Bashir et al. (*Nature* 363, 347, 1993) who demonstrated that activation of mGluRs is necessary for the induction of long-term potentiation (LTP) in nerve cells (septal nucleus, hippocampus) and the finding that long-term depression is induced after activation of mGluRs in cerebellar granule cells (Linden et al. *Neuron* 7, 81, 1991).

[0013] Thus compounds that demonstrate either activating or inhibiting activity at mGluRs have therapeutic potential for the treatment of neurological disorders. These compounds have application as new drugs to treat both acute and chronic neurological disorders, such as stroke and head injuries; epilepsy; movement disorders associated with Parkinson's disease and Huntington's chorea; pain; anxiety; AIDS dementia; and Alzheimer's disease. Since the mGluRs can influence levels of alertness, attention and cognition; protect nerve cells from excitotoxic damage resulting from ischemia, hypoglycemia and anoxia; modulate the level of neuronal excitation; influence central mechanisms involved in controlling movement; reduce sensitivity to pain; and reduce levels of anxiety, these compounds can also be used to influence these situations and also find use in learning and memory deficiencies such as senile dementia. mGluRs may also be involved in addictive behaviour, alcoholism, drug addiction, sensitization and drug withdrawal (*Science*, 280:2045, 1998), so compounds acting at mGluRs might also be used to treat these disorders.

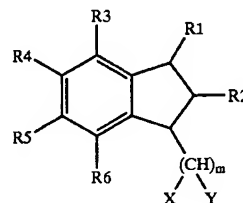
[0014] The current pharmaceutical options for treating neurological disorders tend to be very general and non-specific in their actions in that, although they may reduce the clinical symptoms associated with a specific neurological disorder, they may also negatively impact normal function of the central nervous system of patients. Thus new cellular targets and drugs that are more specific in their actions require to be identified and developed and thus a need remains for chemical compounds that demonstrate specific binding characteristics towards mGluRs.

BRIEF DESCRIPTION OF THE FIGURES

[0015] FIG. 1 demonstrates the actions of compounds of the present invention as antagonists of phosphatidyl inositol hydrolysis evoked through the mGluR1 receptor by 10 μ M (L)-glutamic acid. (650201 corresponds to compound 4b and 650202 corresponds to compound 4a)

SUMMARY OF THE INVENTION

[0016] An object of the present invention is to provide novel aminoindanes that demonstrate activity at the various metabotropic glutamate receptors. In accordance with an aspect of the invention, there is provided a compound of formula (I):



(I)

[0017] stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof, wherein:

[0018] R1, and R2 are selected from the group comprising:

[0019] 1) H; or.

[0020] 2) an acidic group selected from the group comprising carboxy, phosphono, phosphino, sulfono, sulfeno, borono, tetrazol, isoxazol, $-(CH_2)_n$ -carboxy, $-(CH_2)_n$ -phosphono, $-(CH_2)_n$ -phosphino, $-(CH_2)_n$ -sulfono, $-(CH_2)_n$ -sulfeno, $-(CH_2)_n$ -borono, $-(CH_2)_n$ -tetrazol, and $-(CH_2)_n$ -isoxazol, where n = 1, 2, 3, 4, 5, or 6; or

[0021] X is an acidic group selected from the group comprising carboxy, phosphono, phosphino, sulfono, sulfeno, borono, tetrazol, isoxazol;

[0022] Y is a basic group selected from the group comprising 1° amino, 2° amino, 3° amino, quaternary ammonium salts, aliphatic 1° amino, aliphatic 2° amino, aliphatic 3° amino, aliphatic quaternary ammonium salts, aromatic 1° amino, aromatic 2° amino, aromatic 3° amino, aromatic quaternary ammonium salts, imidazol, guanidino, boronoamino, allyl, urea, thiourea;

[0023] m is 0, 1; and

[0024] R3, R4, R5, R6 are independently H, nitro, amino, halogen, tritium, trifluoromethyl, trifluoroacetyl, sulfo, carboxy, carbamoyl, sulfamoyl or pharmaceutically acceptable esters or salts thereof

DETAILED DESCRIPTION OF THE INVENTION

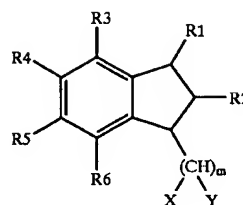
[0025] The terms and abbreviations used in the instant examples have their normal meanings unless otherwise designated. For example "° C." refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "ml" means milliliter or milliliters; "M" refers to molar or molarity; "p-" refers to para, "MS" refers to mass spectrometry; "IR" refers to infrared spectroscopy; and "NMR" refers to nuclear magnetic resonance spectroscopy.

[0026] As would be understood by the skilled artisan, throughout the synthesis of the compounds of Formula I it may be necessary to employ an amino-protecting group or a carboxy-protecting group in order to reversibly preserve a reactively susceptible amino or carboxy functionality while reacting other functional groups on the compound.

[0027] Examples of such amino-protecting groups include formyl, trityl, phthalimido, trichloroacetyl, chloroacetyl, bromoacetyl, iodoacetyl, and urethane-type blocking groups such as benzyloxycarbonyl, 4-phenylbenzyloxycarbonyl, 2-methylbenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 4-fluorobenzyloxycarbonyl, 4-chlorobenzyloxycarbonyl, 3-chlorobenzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, 4-bromobenzyloxycarbonyl, 3-bromobenzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 4-cyanobenzyloxycarbonyl, t-butoxycarbonyl, 2-(4-xenyl)-isopropoxycarbonyl, 1,1-diphenyleth-1-yloxycarbonyl, 1,1-diphenylprop-1-yloxycarbonyl, 2-phenylprop-2-yloxycarbonyl, 2-(p-toluy)-prop-2-yloxycarbonyl, cyclopentanyloxy-carbonyl, 1-methylcyclopentanyloxycarbonyl, cyclohexanyloxycarbonyl, 1-methylcyclohexanyloxycarbonyl, 2-methylcyclohexanyloxycarbonyl, 2-(4-toluy)sulfonyl)-ethoxycarbonyl, 2-(methylsulfonyl)ethoxycarbonyl, 2-(triphenylphosphino)-ethoxycarbonyl, fluorenylmethoxycarbonyl ("Fmoc"), 2-(trimethylsilyl)ethoxycarbonyl, allyloxycarbonyl, 1-(trimethylsilylmethyl)prop-1-enyloxycarbonyl, 5-benzisoxalyl-methoxycarbonyl, 4-acetoxybenzyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-ethynyl-2-propoxycarbonyl, cyclopropylmethoxycarbonyl, 4-(decyloxy)benzyloxycarbonyl, isobornyloxycarbonyl, 1-piperidylloxycarbonyl and the like; benzoylmethylsulfonyl group, 2-nitrophenylsulfonyl, diphenylphosphine oxide and like amino-protecting groups. The species of amino-protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) on other positions of the intermediate molecule and can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other amino-protecting group(s). Preferred amino-protecting groups are t-butoxycarbonyl (t-Boc), allyloxycarbonyl and benzyloxycarbonyl (CbZ). Further examples of these groups are found in E. Haslam, *Protecting Groups in Organic Chemistry*, (J. G. W. McOmie, ed., 1973), at Chapter 2; and T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, (1991), at Chapter 7.

[0028] Examples of such carboxy-protecting groups include methyl, p-nitrobenzyl, p-methylbenzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl, 2,4,6-trimethylbenzyl, pentamethylbenzyl, 3,4-methylenedioxybenzyl, benzhydryl, 4,4'-dimethoxybenzhydryl, 2,2',4,4'-tetramethoxybenzhydryl, t-butyl, t-amyl, trityl, 4-methoxytrityl, 4,4'-dimethoxytrityl, 4,4',4''-trimethoxytrityl, 2-phenylprop-2-yl, trimethylsilyl, t-butyl dimethylsilyl, phenacyl, 2,2,2-trichloroethyl, β -(di(n-butyl)methylsilyl)ethyl, p-toluenesulfonylethyl, 4-nitrobenzylsulfonylethyl, allyl, cinnamyl, 1-(trimethylsilylmethyl)prop-1-en-3-yl and like moieties. Preferred carboxy-protecting groups are allyl, benzyl and t-butyl. Further examples of these groups are found in E. Haslam, *supra*, at Chapter 5; and T. W. Greene and P. G. M. Wuts, *supra*, at Chapter 5.

[0029] The present invention provides a compound of the formula I:



(I)

[0030] Stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof, wherein:

[0031] R1, and R2 are selected from the group comprising:

[0032] 1) H

[0033] 2) an acidic group selected from the group comprising carboxy, phosphono, phosphino, sulfono, sulfinio, borono, tetrazol, isoxazol, $-(CH_2)_n$ -carboxy, $-(CH_2)_n$ -phosphono, $-(CH_2)_n$ -phosphino, $-(CH_2)_n$ -sulfono, $-(CH_2)_n$ -sulfinio, $-(CH_2)_n$ -borono, $-(CH_2)_n$ -tetrazol, and $-(CH_2)_n$ -isoxazol, where $n=1, 2, 3, 4, 5$, or 6 ; or

$n=1, 2, \dots$

[0034] X is an acidic group selected from the group comprising carboxy, phosphono, phosphino, sulfono, sulfinio, borono, tetrazol, isoxazol;

[0035] Y is be a basic group selected from the group comprising 1° amino, 2° amino, 3° amino, quaternary ammonium salts, aliphatic 1° amino, aliphatic 2° amino, aliphatic 3° amino, aliphatic quaternary ammonium salts, aromatic 1° amino, aromatic 2° amino, aromatic 3° amino, aromatic quaternary ammonium salts, imidazol, guanidino, boronoamino, allyl, urea, thiourea;

[0036] m can be 0, 1; and

[0037] R3, R4, R5, R6 are independently H, nitro, amino, halogen, tritium, trifluoromethyl, trifluoroacetyl, sulfo, carboxy, carbamoyl, sulfamoyl or pharmaceutically acceptable esters or salts thereof

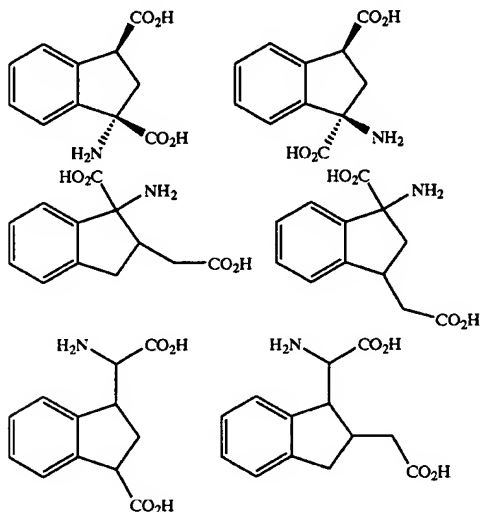
acetyl, sulfo

[0038] In one embodiment of the present invention a compound of formula (I) is provided, wherein: R1 is CO_2H , or CH_2CO_2H ; R2 is H; X is CO_2H ; and Y is NH_2

[0039] In another embodiment of the present invention a compound of formula (I) is provided, wherein:

[0040] R1 is H; R2 is CO_2H or CH_2CO_2H ; X is CO_2H ; and Y is NH_2 .

[0041] Compounds of the present invention include, but are not limited to the following exemplary molecules:



[0042] While all of the compounds of Formula I are believed to demonstrate activity at the metabotropic glutamate receptors (mGluRs), certain groups of Formula I compounds are more preferred for such use.

[0043] As noted supra, this invention includes the pharmaceutically acceptable salts of the compounds defined by Formula I. A compound of this invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with any of a number of organic and inorganic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt.

[0044] The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds of the above formula which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a pharmaceutically acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts.

[0045] Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogen phosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, hydrochloride, dihydrochloride, isobutyrate, caproate, heptanoate, propionate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropi-

onate, phenylbutyrate, citrate, lactate, γ-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like.

[0046] Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulfonic acid.

ammonium

[0047] Salts of amine groups may also comprise quaternary ammonium salts wherein the amino nitrogen carries a suitable organic group such as an alkyl, alkenyl, alkynyl, or aralkyl moiety.

[0048] Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred.

[0049] It should be recognized that the particular counterion forming a part of any salt of this invention is usually not of a critical nature, as long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

[0050] This invention further encompasses the pharmaceutically acceptable solvates of the compounds of Formula I. Many of the Formula I compounds can combine with solvents such as water, methanol, ethanol and acetonitrile to form pharmaceutically acceptable solvates such as the corresponding hydrate, methanolate, ethanolate and acetonitrilate.

[0051] The compounds of the present invention have multiple asymmetric (chiral) centers. As a consequence of these chiral centers, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All asymmetric forms, individual isomers and combinations thereof, are within the scope of the present invention.

[0052] The prefixes "R" and "S" are used herein as commonly used in organic chemistry to denote the absolute configuration of a chiral center, according to the Cahn-Ingold-Prelog system. The stereochemical descriptor R (rectus) refers to that configuration of a chiral center with a clockwise relationship of groups tracing the path from highest to second-lowest priorities when viewed from the side opposite to that of the lowest priority group. The stereochemical descriptor S (sinister) refers to that configuration of a chiral center with a counterclockwise relationship of groups tracing the path from highest to second-lowest priority when viewed from the side opposite to the lowest priority group. The priority of groups is decided using sequence rules as described by Cahn et al., *Angew. Chem.*, 78, 413-447, 1966 and Prelog, V. and Helmchen, G.; *Angew. Chem. Int. Ed. Engl.*, 21, 567-583, 1982).

[0053] In addition to the R,S system used to designate the absolute configuration of a chiral center, the older D-L

monohydrogen phosphate

protecting group, or a salt thereof, and R11 is a hydrogen atom or a nitrogen protecting group;

[0067] whereafter, if necessary and/or desired, the following steps are carried out:

[0068] (i) resolving the compound of Formula I;

[0069] (ii) converting the compound of Formula I into a non-toxic metabolically labile ester or amide thereof, and/or;

[0070] (iii) converting the compound of Formula I or a non-toxic metabolically labile ester or amide thereof into a pharmaceutically acceptable salt thereof.

[0071] Compounds of formulae (II), (III) and (IV), wherein at least one of R3, R4, R5 and R6 is other than H may be prepared from the compounds of formula (II), (III) and (IV) respectively, wherein: R3, R4, R5 and R6 is H, using standard reactions known to a person skilled in the art. For example: electrophilic substitution with appropriate electrophile, Friedel-Crafts alkylation or acylation, followed by further manipulations of the formed products within the knowledge of a worker skilled in the art.

[0072] The protection of carboxylic acid and amine groups is generally described in McOmie, *Protecting Groups in Organic Chemistry*, Plenum Press, N.Y., 1973, and Greene and Wuts, *Protective Groups in Organic Synthesis*, 2nd. Ed., John Wiley & Sons, NY, 1991. Examples of carboxy protecting groups include alkyl groups such as methyl, ethyl, t-butyl and t-amyl; aralkyl groups such as benzyl, 4-nitrobenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl, 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl, 2,4,6-trimethylbenzyl, benzhydryl and trityl; silyl groups such as trimethylsilyl and t-butyltrimethylsilyl; and allyl groups such as allyl and 1-(trimethylsilylmethyl)prop-1-en-3-yl. Examples of amine protecting groups include acyl groups, such as groups of formula $-C(O)R_{11}$ in which R11 represents (C₁-C₆) alkyl, (3-10C) cycloalkyl, phenyl (C₁-C₆) alkyl, phenyl (C₁-C₆) alkoxy, or a (C₃-C₁₀) cycloalkoxy, wherein a phenyl group may optionally be substituted by one or two substituents independently selected from amino, hydroxy, nitro, halogeno, (C₁-C₆) alkyl, (C₁-C₆) alkoxy, carboxy, (C₁-C₆) alkoxy-carbonyl, carbamoyl, (C₁-C₆) alkanoylamino, (C₁-C₆) alkylsulphonylamino, phenylsulphonylamino, toluenesulphonylamino, and (C₁-C₆) fluoroalkyl.

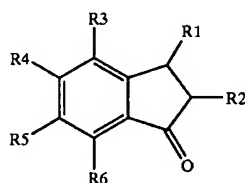
[0073] The compounds of Formula II are conveniently hydrolyzed in the presence of an acid, such as hydrochloric acid or sulfuric acid, or a base, such as an alkali metal hydroxide, for example sodium hydroxide. The hydrolysis is conveniently performed in an aqueous solvent such as water and at a temperature in the range from 50 to 200 ° C.

[0074] The compounds of Formula III are conveniently hydrolyzed in the presence of a base, for example an alkali metal hydroxide such as lithium, sodium or potassium hydroxide, or an alkaline earth metal hydroxide such as barium hydroxide. Suitable reaction media include water. The temperature is conveniently in the range from 50 to 150 ° C.

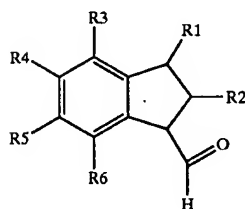
[0075] The compounds of Formula IV may be deprotected by conventional methods. Thus, an alkyl carboxyl protecting group may be removed by hydrolysis. The hydrolysis may

conveniently be performed by heating the compound of Formula IV in the presence of either a base, for example an alkali metal hydroxide such as lithium, sodium or potassium hydroxide, or an alkaline metal hydroxide, such as barium hydroxide, or an acid such as hydrochloric acid. The hydrolysis is conveniently performed at a temperature in the range from 10 ° to 300 ° C. An aralkyl carboxyl-protecting group may conveniently be removed by hydrogenolysis. The hydrogenolysis may conveniently be effected by reacting the compound of Formula IV with hydrogen in the presence of a Group VIII metal catalyst, for example a palladium catalyst such as palladium on charcoal. Suitable solvents for the reaction include alcohols such as ethanol. The reaction is conveniently performed at a temperature in the range from 0 ° to 100 ° C. An acyl amine protecting group is also conveniently removed by hydrolysis, for example as described for the removal of an alkyl carboxyl protecting group.

[0076] The compounds of Formula (IIa) and (IIb) may be prepared by reacting compounds of formula (Va) and (Vb) respectively:



(Va)

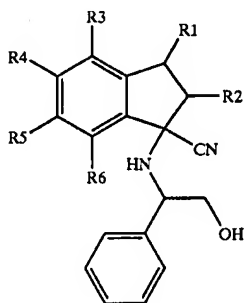


(Vb)

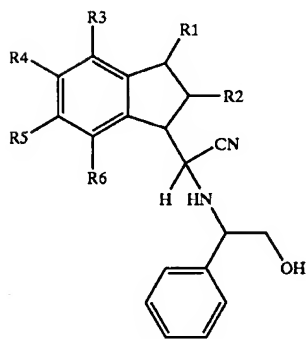
[0077] wherein: R1, R2, R3, R4, R5 and R6 are as defined above; with an alkali metal cyanide, such as lithium, sodium or potassium cyanide, and either ammonium carbonate in an aqueous alcohol, such as aqueous ethanol or with an ammonium halide, such as ammonium chloride, conveniently in the presence of ultrasound. If the reaction is conducted with ammonium carbonate, the reaction is conveniently performed at a temperature in the range from 35° C. to 150° C. If desired, the compounds of Formula II may then be alkylated, for example using a compound of formula RCl, wherein: R is (C₁-C₆) straight or branched chain alkyl, or (C₁-C₆) alkanoyl group. As described in more detail hereinafter, the alkylated compounds may be readily separated into their diastereomers. If the reaction is conducted with an ammonium halide in the presence of ultrasound, the ammonium halide is mixed with chromatography grade alumina in the presence of a suitable diluent such as acetonitrile. The

mixture is then irradiated with ultrasound, whereafter the compound of Formula V is added, and the mixture is again irradiated. The alkali metal cyanide is then added, followed by further irradiation with ultrasound.

[0078] Individual isomers of compounds of Formula (IIa) and (IIb) may be made by reacting a compound of the Formula V with the stereoisomers of the chiral agent (S)- and (R)-phenylglycinol and a reactive cyanide such as trimethylsilyl cyanide to form the intermediate compounds of Formula (VIa) or (VIb), that can be further hydrolysed to give the desired products.



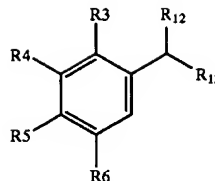
(VIa)



(VIb)

[0079] The compounds of Formula III may be prepared by reacting a compound of Formula V with an alkali metal cyanide, such as lithium, sodium or potassium cyanide, and ammonium carbonate or ammonium carbamate. Common solvents include alcohols, such as methanol, aqueous methanol and aqueous ethanol. Conveniently the reaction is performed at a temperature in the range of from 10° to 150° C. If desired, the compounds of Formula (m) may then be alkylated, for example using an appropriate alkyl, aryl or acyl chloride.

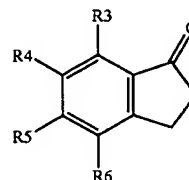
[0080] The compounds of Formula (Va) are either commercially available or may be prepared using standard procedures known to a person skilled in the relevant art. For example: compounds of formula (Va) can be prepared by reacting a compound of Formula VII with thionyl chloride or phosphorus pentachloride, and subjecting the resulting compound to the Friedel-Crafts acylation conditions.



VII

[0081] wherein: R12 and R13 are together or independently CO₂H or CH₂CO₂H

[0082] In an alternative manner the compounds of formula (Va) can be prepared from compound VIII, by alkylation, followed by hydrolysis of the resulting compounds.



VIII

[0083] Compounds of formulae (VII), and (VII) are either commercially available or may be prepared using standard procedures known to a person skilled in the art. Compounds of formulae (VII), and (VII) wherein at least one of R3, R4, R5 and R6 is other than H may be prepared from the compounds of formulae (VII), and (VII) respectively, wherein R3, R4, R5 and R6 is H, via standard reactions known to a person skilled in the art. For example: electrophilic substitution with appropriate electrophile, Friedel-Crafts alkylation or acylation, followed by further manipulations of the formed products within the knowledge of a worker skilled in the art.

[0084] The compounds of formula (Vb) may be prepared by reacting a compound of formula (Va) with Wittig salt such as (alkoxy methyl)triphenylphosphonium halide in the presence of alkali metal salt of bis (trimethylsilyl) amine or by reacting a compound of formula (Va) with Wittig reagent such as Ph₃P=CHOCH₃, followed by reaction with trimethylsilyl halide.

[0085] Compounds of formulae (Va), and (Vb), wherein at least one of R3, R4, R5 and R6 is other than H may be prepared from the compounds of formula (Va) and (Vb) respectively, wherein R3, R4, R5 and R6 is H, using standard reactions known to a person skilled in the art. For example: electrophilic substitution with appropriate electrophile, Friedel-Crafts alkylation or acylation, followed by further manipulations of the formed products within the knowledge of a worker skilled in the art.

[0086] In an alternative manner, compounds of formula (V) may be prepared as taught in U.S. Pat. Nos.: 5,329,049; and 5,360,936.

[0087] Many of the intermediates described herein, for example the compounds of Formula II, III and IV are believed to be novel, and are provided as further aspects of the invention.

[0088] Biological and Therapeutic Activity of Compounds of Formula I

[0089] The compounds of formula I of the present invention exhibit agonists or antagonists activity toward certain metabotropic glutamate receptors (mGluRs). Therefore, another aspect of the present invention provides a method of modulating the activity of mGluRs in mammals, which comprises administering to a mammal requiring modulated excitatory amino acid neurotransmission a pharmacologically-effective amount of a compound of Formula I. The term "pharmacologically-effective amount" is used to represent an amount of the compound of the present invention that is capable of affecting the mGluRs. By modulating mGluR activity, a compound of the present invention is acting as an agonist or antagonist of mGluR. When a compound of the present invention acts as an agonist, the interaction of the compound with the excitatory amino acid receptor mimics the response of the interaction of this receptor with its natural ligand, (i.e. L-Glutamic acid). When a compound of the invention acts as an antagonist, the interaction of the compound with the excitatory amino acid receptor blocks or attenuates the response of the interaction of this receptor with its natural ligand, (i.e. L-Glutamic acid).

[0090] The particular dose of compound administered according to the present invention will, of course, be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and similar considerations. The compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, or intranasal routes. Alternatively, the compound may be administered by continuous infusion. A typical daily dose will contain from about 0.001 mg/kg to about 100 mg/kg of the active compound of this invention. Preferably, daily doses will be about 0.05 mg/kg to about 50 mg/kg, more preferably from about 0.1 mg/kg to about 20 mg/kg. A variety of physiological functions have been shown to be subject to influence by excessive or inappropriate stimulation of excitatory amino acid transmission. The Formula I compounds of the present invention are believed (through their interactions at the mGluRs) to have the ability to treat a variety of neurological disorders in a warm-blooded mammals associated with abnormal excitatory amino acid transmission, including but not limited to acute neurological disorders such as cerebral deficits subsequent to cardiac bypass surgery and grafting, cerebral ischemia (e.g. stroke and cardiac arrest), spinal cord trauma, head trauma, perinatal hypoxia, and hypoglycemic neuronal damage. Similarly, the Formula I compounds of the present invention, through their modulation of mGluR activity are believed to have the ability to treat a variety of chronic neurological disorders, such as Alzheimer's disease, Huntington's Chorea, amyotrophic lateral sclerosis, AIDS-induced dementia, ocular damage and retinopathy, cognitive disorders, and idiopathic and drug-induced Parkinson's disease. The present invention also provides methods for treat-

ing these disorders which comprises administering to a patient in need thereof an effective amount of a compound of Formula I.

[0091] The Formula I compounds of the present invention, through their modulation of mGluR activity are also believed to have the ability to treat a variety of other neurological disorders in mammals that are associated with glutamate dysfunction, including muscular spasms, convulsions, migraine headaches, urinary incontinence, psychosis, drug tolerance, withdrawal, and cessation (i.e. opiates, benzodiazepines, nicotine, cocaine, or ethanol), smoking cessation, anxiety and related disorders (e.g. panic attack), emesis, brain edema, chronic pain, sleep disorders, Tourette's syndrome, attention deficit disorder, and tardive dyskinesia. Therefore, the present invention also provides methods for treating these disorders which comprise administering to a patient in need thereof an effective amount of the compound of Formula I.

[0092] The Formula I compounds of the present invention, through their modulation of mGluR activity are also believed to have the ability to treat a variety of psychiatric disorders, such as schizophrenia, anxiety and related disorders (e.g. panic attack), depression, bipolar disorders, psychosis, and obsessive compulsive disorders. The present invention also provides methods for treating these disorders which comprises administering to a patient in need thereof an effective amount of a compound of Formula I.

[0093] Functional Assays Employing Cloned Subtypes of Metabotropic Receptors

[0094] The pharmacological properties of the compounds of the present invention can be determined via appropriate functional assays using recombinant metabotropic glutamate receptors. For example adenylate cyclase assays or phosphatidylinositol hydrolysis assays, performed using standard procedures, can be used to determine agonist or antagonist activity towards mGluRs.

[0095] In Vitro Testing. *in vitro*

[0096] General *in vitro* assay methods include monitoring of adenylate cyclase activity and phosphatidyl inositol hydrolysis in a cell line that expresses the appropriate mGluR.

[0097] (a) Adenylate Cyclase Activity

[0098] Adenylate cyclase activity is determined in initial experiments in transfected mammalian cells, using standard techniques. See, e.g., N. Adham, et al., *Supra*; R L. Weinshank, et al. *Proc. Natl. Acad. Sci. (USA)*, 89:3630-3634 (1992), and the references cited therein.

[0099] Mammalian cells (the cell line AV12-664 is especially preferred) are stably transfected with a plasmid comprising the cloned metabotropic glutamate receptor. The cells are maintained in an appropriate medium, for example one consisting of Dulbecco's Modified Eagle's Medium (DMEM) containing 5% dialyzed fetal calf serum, 10 mM HEPES buffer (pH 7.3), 1 mM sodium pyruvate, 1 mM glutamine, and 200 μ g/mL hygromycin. *mM*

[0100] For the assay the cells are disassociated from stock culture flasks with trypsin, and plated in 24-well plastic tissue culture dishes (15 mm wells) at a density of 500,000-700,000 cells per well using the same culture medium. After

a twenty four hour incubation in a humidified CO₂ incubator, the cell monolayers are washed with buffer (for example Dulbecco's phosphate-buffered saline containing 0.5 mM IBMX and 3 mM glucose) and then incubated in the same buffer at 37° C. for 30 minutes. The monolayers are then washed with six exchanges of buffer.

[0101] Test compound(s) and forskolin, or forskolin alone, dissolved in buffer, are added after the final wash. After incubating for 20 minutes at 37° C., 0.5 mL of 8 mM EDTA is added to each well. The plates are then placed in a boiling water bath for about four minutes. The supernatant fluids are recovered from the wells and lyophilized. Cyclic AMP (cAMP) determinations are carried out on the lyophilized samples using commercially available radioimmunoassay kits, following the manufacturer's instructions. The cAMP levels in wells containing test compound(s) are then compared to the forskolin controls.

[0102] (b) Phosphatidylinositol Assay

[0103] Phosphatidylinositol hydrolysis is measured in clonal cell lines (for example AV12) harbouring a plasmid expressing the cloned metabotropic glutamate receptor in response to addition of glutamate agonists, as a functional assay for metabotropic glutamate receptor activity according to D. Schoepp, Trends in Pharmaceutical Sciences, 11:508, 1990.

[0104] Twenty four well tissue culture vessels are seeded with approximately 250,000 cells per well in an appropriate medium for example Dulbecco's Minimal Essential Media (D-MEM) (in the absence of glutamic acid) containing 2 mM glutamine and 10% dialyzed fetal calf serum. After 24 hours growth at 37° C., the media is removed and replaced with fresh media containing four microcuries of [³H]myo-inositol per well and the cultures are incubated a further 16 to 20 hours. The media is then removed and the cells in each well are washed with serum free medium containing 10 mM lithium chloride, 10 mM myo-inositol, and 10 mM HEPES (2x1 mL washes). After the final wash, 0.5 mL of washing solution is added containing the appropriate concentration(s) of test compound(s).

[0105] If the particular assay is also testing antagonists, a ten minutes incubation is performed prior to antagonist induction. Cells are incubated for about one hour at 37° C. in 95%:5% O₂: CO₂ or as appropriate for time course. The reactions are terminated by removing media and adding 1 mL of cooled 1:1 acetone:methanol followed by incubation on ice for a minimum of twenty minutes.

[0106] These extracts are then collected and placed in 1.5 mL centrifuge tubes. Each well is washed with 0.5 mL water and this wash is added to the appropriate extract. After mixing and centrifugation, each aqueous supernatant is processed by chromatography on a QMA SEP-PAK® column, which is prewetted and equilibrated by passing 10 mL of water, followed by 8 mL of 1M triethylammonium hydrogen carbonate (TEAB), followed by 10 mL of water through the column.

[0107] The assay supernatants, containing the water soluble [³H]inositol phosphate are passed over the columns. This is followed by a 10 mL water wash and a 4 mL wash with 0.02 M TEAB to remove [³H]inositol precursors. [³H]inositol phosphate is eluted with 4 mL of 0.1 M TEAB into scintillation vials and counted in the presence of scin-

tillation cocktail. Total protein in each sample is measured using standard techniques. Measurements are taken as the amount of [³H]inositol phosphate released per milligram of protein.

[0108] The assays are carried out in the absence and in the presence of the compound being tested. The measurements of [³H]inositol phosphate per milligram of protein are compared in order to confirm agonist and antagonist activity of the compound being tested.

[0109] These types of assays, employing cell lines expressing different subtype of cloned metabotropic receptors, may be used to determine which compounds have selective affinity in that they modulate one subtype of receptor with a greater activity than another subtype.

[0110] (c) Testing in Chinese Hamster Cell Lines

[0111] The Chinese hamster ovary cell lines expressing mGluR_{1α}, mGluR₂ and mGluR_{4α} receptors have been described previously (Amarori and Nakanishi, Neuron 8, 757-765, 1992; Tanabe et al., Neuron 8, 169-179, 1992, and J. Neurochem. 63, 2038-2047, 1993). They are maintained at 37° C. in a humidified 5% CO₂ incubator in Dubecco's Modified Eagle Medium (DMEM) containing a reduced concentration of (S)-glutamine (2 mM) and are supplemented with 1% proline, penicillin (100 U/mL), streptomycin (100 mg/mL) and 10% dialyzed fetal calf serum (all GIBCO, Paisley). Two days before assay 1.8x10⁶ cells are evenly distributed into the wells of 24 well plates.

[0112] Phosphatidylinositol (PI) hydrolysis can be measured as described previously (Hayashi et al., Nature 366, 687-690, 1992, and J. Neuroscience 14, 3370-3377, 1994). Briefly, the cells are labeled with [³H]inositol (2 μCi/mL) 24 h prior to the assay. For agonist assays, the cells are incubated with test compound dissolved in phosphate-buffered saline (PBS)-LiCl for 20 min, and agonist activity is determined from the level of ³H-labeled mono-, bis- and tris-inositol phosphates generated, as measured following ion-exchange chromatography, compared with the level generated in the absence of the test compound. For antagonist assays, the cells are preincubated with ligand dissolved in PBS-LiCl for 20 min prior to incubation with test compound and 10 μM (S)-Glu for 20 min. The antagonist activity is then determined as the inhibitory effect of the (S)-Glu mediated response.

[0113] The assay of cyclic AMP formation can be performed as described previously (Hayashi et al., 1992, 1994). Briefly, the cells are incubated for 10 min in PBS containing test compound and 10 μM forskolin and 1 mM 3-isobutyl-1-methylxanthine (IBMX) (both Sigma, St. Louis, Mo., USA). The agonist activity is then determined as the inhibitory effect on the forskolin-induced cyclic AMP formation. For antagonist assay, the cells are preincubated with ligand dissolved in PBS containing 1 mM (IBMX) for 20 min prior to a 10 min incubation in PBS containing test compound, 20 μM (mGlu2) or 50 μM (mGlu4a) (S)-Glu, 10 μM forskolin and 1 mM IBMX. The antagonist activity is then determined as the potentiating effect on the forskolin-induced cyclic AMP formation.

[0114] Some of the compounds of the invention were tested for antagonist activity against Chinese hamster ovary cell lines expressing cloned mGluR_{1α}, mGluR₂ and mGluR_{4α} at a concentration of 5 mM. Compounds 4a and 4b

[³H]inositol

of the invention effectively blocked the increase in PI hydrolysis by action of 10 mM (L)-glutamic acid at the mGluR_{1α} receptor. The results are presented in FIG. 1.

[0115] In Vivo Testing:

[0116] In vivo testing for demonstration of the pharmacological activity of certain compounds on representative mGlu receptor subtypes can be performed using Sprague Dawley rat tissues.

[0117] Phosphatidylinositol (PI) hydrolysis can be measured as described below:

[0118] Briefly, cross-chopped slices are prepared from neonatal Sprague Dawley rat tissue (age: p7-p14). The slices are pre-labelled with ³H-myoinositol. Following pre-labelling, the slices are incubated with the test drugs and standard (known Group I agonists i.e. ACPD) for a period of 45 minutes. The incubation is terminated by the addition of chloroform/methanol/HCl (100:200:2). The resulting mixture is separated into two phases by the addition of chloroform and distilled water. The aqueous fraction is applied to ion exchange columns, and inositol phosphates are eluted using 800 mM Ammonium Formate/100 mM Formic Acid. The eluent is then analyzed using liquid scintillation counting. The amount of inositol phosphate accumulation is expressed as a percentage of that induced by ACPD.

[0119] The assay of cyclic AMP formation can be performed as described previously (Tovey et al., *Clinica Chimica Acta*, 56, 221-234, 1974). The assay can be modelled on the cyclic AMP assay kit available from Amersham, which in turn, is based on the assay created by Tovey et al. Briefly, samples are prepared from Sprague Dawley rat (225-250 g) cortical slices. Slices are incubated with the drug, and then challenged with forskolin to induce cyclic AMP release. Following termination of the reaction by boiling, the slices are homogenized and centrifuged. Samples of supernatant are then incubated for 2-3 hours with a known quantity of [³H]cAMP and a binding protein. When the incubation is complete, the bound cyclic AMP is separated from the free cyclic AMP by centrifugation with charcoal. The resulting supernatant (containing free cyclic AMP) is then analyzed by liquid scintillation counting. The amount of unbound cyclic AMP can be calculated from a standard curve previously determined with various samples of free cyclic AMP.

[0120] In performing such experiments with some of the compounds of the present invention, it has been demonstrated that some compounds of the present invention act as modulators of the cAMP-linked metabotropic glutamate receptors, while showing less activity with phosphatidylinositol-linked metabotropic glutamate receptors and vice versa.

[0121] Administration of compounds of Formula (I)

[0122] According to another aspect, the present invention provides a method of modulating one or more mGluR functions in a warm-blooded mammal which comprises administering an effective amount of a compound of Formula I, or a non-toxic metabolically-labile ester or amide thereof, or a pharmaceutically acceptable salt thereof.

[0123] The compounds of the present invention are preferably formulated prior to administration. Therefore, another aspect of the present invention is a pharmaceutical formu-

lation comprising a compound of Formula I and a pharmaceutically-acceptable carrier, diluent, or excipient. The present pharmaceutical formulations are prepared by known procedures using well-known and readily available ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, and may be in the form of a capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be a solid, semi-solid, or liquid material that acts as a vehicle, excipient, or medium for the active ingredient.

[0124] The compounds of Formula I are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

[0125] The present invention also provides pharmaceutical compositions containing compounds as disclosed in the claims in combination with one or more pharmaceutically acceptable, inert or physiologically active, diluents or adjuvants. The compounds of the invention can be freeze-dried and, if desired, combined with other pharmaceutically acceptable excipients to prepare formulations for administration. These compositions may be presented in any form appropriate for the administration route envisaged. The parenteral and the intravenous route are the preferential routes for administration.

[0126] Compounds of the general Formula I may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition, there is provided a pharmaceutical formulation comprising a compound of general Formula I and a pharmaceutically acceptable carrier. One or more compounds of general Formula I may be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants and, if desired, other active ingredients. The pharmaceutical compositions containing compounds of general Formula I may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs.

[0127] Compositions intended for oral use may be prepared according to any procedure known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents for example, corn

Aug. 5, 2004

of about 0.1 to about 15 mg/kg/day, in single or divided dose, is especially preferred. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several smaller doses for administration throughout the day.

[0138] The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 mg to about 500 mg, more preferably about 25 mg to about 300 mg of the active ingredient. The term "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

Formulation 1

[0139] Hard gelatin capsules are prepared using the following ingredients:

| | Quantity (mg/capsule) |
|--------------------|-----------------------|
| Active Ingredient | 250 |
| Starch, dried | 200 |
| Magnesium stearate | 10 |
| Total | 460 |

Formulation 2

[0140] A tablet is prepared using the ingredients below:

| | Quantity (mg/tablet) |
|-----------------------------|----------------------|
| Active Ingredient | 250 |
| Cellulose, microcrystalline | 400 |
| Silicon dioxide, fumed | 10 |
| Stearic acid | 5 |
| Total | 665 |

Formulation 3

[0141] An aerosol solution is prepared containing the following components:

| | Weight % |
|---------------------------------------|----------|
| Active Ingredient | 0.25 |
| Ethanol | 29.75 |
| Propellant 22 (Chlorodifluoromethane) | 70.00 |
| Total | 100 |

[0142] The active compound is mixed with ethanol and the mixture added to a portion of the Propellant 22, cooled to -30° C. and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

[0143] Tablets each containing 60 mg of active ingredient are made as follows:

| | Quantity (mg/tablet) |
|-----------------------------|----------------------|
| Active Ingredient | 60 |
| Starch | 45 |
| Microcrystalline cellulose | 35 |
| Polyvinylpyrrolidone | 4 |
| Sodium carboxymethyl starch | 4.5 |
| Magnesium stearate | 0.5 |
| Talc | 1.0 |
| Total | 150 |

[0144] The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders that are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50° C. and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

[0145] Capsules each containing 80 mg medicament are made as follows:

| | Quantity (mg/capsule) |
|----------------------------|-----------------------|
| Active Ingredient | 80 |
| Starch | 59 |
| Microcrystalline cellulose | 59 |
| Magnesium stearate | 2 |
| Total | 200 |

[0146] The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 45 sieve, and filled into hard gelatin capsules in 200 mg quantities.

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

The components are blended and compressed to form tablets each weighing 665 mg.

trated. The product was purified with column chromatography (hexanes:EtOAc, 8:2) to obtain 0.68 g of compound 21 as a faint yellow oil.

[0201] Preparation of Intermediate Compound (23):

[0202] A mixture of compound 21 (0.65 g), KCN (0.54 g) and $(\text{NH}_4)_2\text{CO}_3$ (2.2319 g) in a solution of EtOH/H₂O (8 mL/8 mL) was stirred in a pressure tube at -100°C . for 3 days. More KCN (0.27 g) and $(\text{NH}_4)_2\text{CO}_3$ (1.11 g) were added to the mixture after 27 hours. The mixture was cooled down to room temperature and acidified with 6 N HCl to pH~2, and the solvents removed by rotary evaporation. MeOH was added to the residue and the resulting suspension was filtered. The filtrate was concentrated and the resulting residue was dissolved in 3 N NaOH (40 mL) and refluxed for 2 days. The obtained crude product was re-dissolved in 2 N NaOH/1,4-dioxane (13 mL:13 mL) and CbzCl (0.67 mL) was added at 0°C ., while stirring. The mixture was stirred for 1 hour at 0°C . and at room temperature for 2 days. More CbzCl was added to the reaction mixture (0.9 mL after 7 hours, 0.5 mL after 1 day) and 2 N NaOH added to adjust the pH to ~9.

[0203] The mixture was extracted with EtOAc (4x50 mL). The aqueous solution was cooled to 0°C ., acidified with 6 N HCl to pH ~2 and extracted with EtOAc (4x150 mL). Combined organic extracts were washed with brine (2x100 mL), dried and concentrated. The crude product was purified by column chromatography (CH_2Cl_2 : Acetone:HCO₂H, 85:15:1) to obtain protected form of compound 23. This product was hydrogenized in 20 mL MeOH with 10% Pd/C (18 mg) under H₂ for 24 hours. The solution was filtered through Celite and crystallized from MeOH to obtain 82.4 mg of compound 23 as an off-white solid. Elemental analysis calc. for compound 23: C; 60.12, H; 5.68, N; 5.84. Found: C; 60.11, H; 5.55, N; 5.79. ¹H NMR (D₂O) δ 2.2 (t, 2H), 3.6-3.8 (m, 2H due to overlapping single protons), 3.9 (d, 1H), 7.3 (s, 4H).

Example 6

[0204] In Vivo Testing of Exemplary Compounds:

[0205] Cyclic AMP Assay:

[0206] Rationale:

[0207] Group II/III metabotropic glutamate receptors (mGluRs) are negatively coupled to adenylate cyclase, and agonists of these receptors lead to a decrease in intracellular cyclic AMP accumulation.

[0208] Method:

[0209] The assay has been modeled on the cyclic AMP assay kit available from Amersham. This kit, in turn, is based on the assay created by Tovey et al. (1974). Briefly, the samples were prepared from Sprague Dawley rat (225-250 g) cortical slices. Slices were incubated with the test compound (drug), and then challenged with forskolin to induce cyclic AMP release. Following termination of the reaction by boiling, the slices were homogenized and centrifuged. Samples of supernatant were then incubated for 2-3 hours with a known quantity of [³H]cAMP and a binding protein. When the incubation was complete, the bound cyclic AMP was separated from the free cyclic AMP by centrifugation with charcoal. The resulting supernatant (containing free cyclic AMP) was then analyzed by liquid scintillation count-

ing. The amount of unbound cyclic AMP was calculated from a standard curve previously determined with various samples of free cyclic AMP.

[0210] Results Interpretation:

[0211] If the drugs tested inhibit forskolin-induced cyclic AMP accumulation, they are considered to be Group II/III agonists. Conversely, if they inhibit the decrease in forskolin-induced cyclic AMP accumulation caused by glutamate, they are considered to be Group II/III antagonists.

[0212] Results:

| Aminoindane | Group II/III Agonist | EC ₅₀ (M) | Group II/III Antagonist | EC ₅₀ (M) |
|---|----------------------|----------------------|-------------------------|----------------------|
| Trans-1-aminoindane-1,3-dicarboxylic acid Compound 4a | No | — | No | — |
| Cis-1-aminoindane-1,3-dicarboxylic acid Compound 4b | Yes | 2.1×10^{-6} | No | — |
| Trans-1-amino-1-carboxyindane-2-acetic acid Compound 9 | No | — | Yes | 3.4×10^{-8} |
| Trans-1-amino-1-carboxyindane-3-acetic acid Compound 13 | Yes | 9.9×10^{-7} | No | — |
| 1-[2-amino]-2-indanediacetic acid Compound 18 | Yes | 4.6×10^{-7} | Yes | 2.6×10^{-8} |
| 1-[2-amino]-3-carboxyindaneacetic acid Compound 23 | No | — | Yes | 6.1×10^{-7} |

[0213] Phosphatidylinositol Assay

[0214] Rationale:

[0215] Group I metabotropic glutamate receptors (mGluRs) are positively coupled on inositol phosphate metabolism. Agonists at these receptors lead to an increase in intracellular free inositol phosphates, while antagonists inhibit the increase in intracellular free inositol phosphate induced by standard agonists (i.e. ACPD).

[0216] Method:

[0217] Cross-chopped slices were prepared from neonatal Sprague Dawley rat tissue (age: p7-p14). The slices were pre-labelled with [³H] myo-inositol. Following pre-labeling, the slices were incubated with the test compounds and standard (known Group I agonists i.e. ACPD) for a period of 45 minutes. The incubation was terminated by the addition of chloroform/methanol/HCl (100:200:2). The resulting mixture was separated into two phases by the addition of chloroform and distilled water. The aqueous fraction was applied to ion exchange columns, and inositol phosphates were eluted using 800 mM Ammonium Formate/100 mM Formic Acid. The eluent was then analyzed using liquid scintillation counting. The amount of inositol phosphate accumulation was expressed as a percentage of that induced by ACPD.